Polymorphisms analysis of *CYP21A2* gene associated with Congenital Adrenal Hyperplasia

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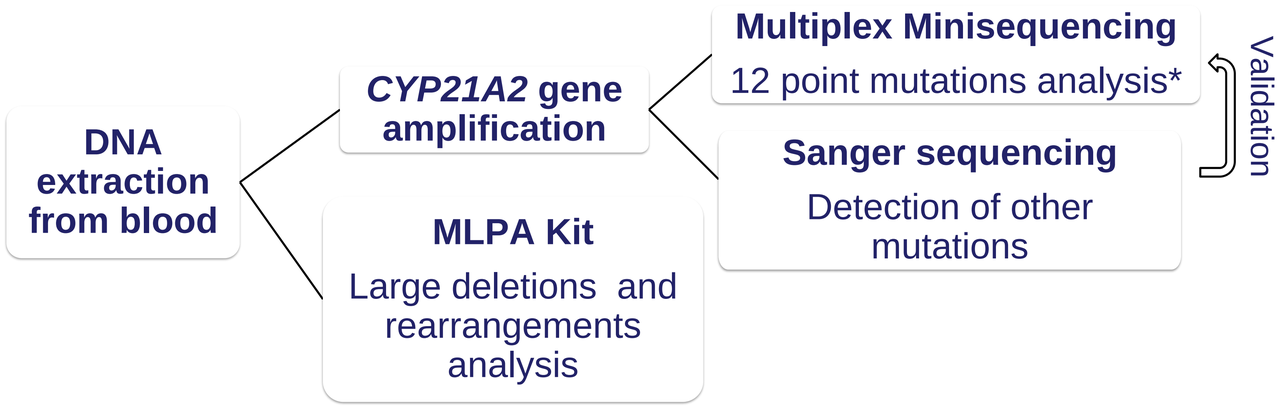
INTRODUTION

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders of adrenal steroidogenesis caused by a genetic disorder in one of the enzymes involved in cortisol biosynthesis. In 90% of cases, CAH is due to 21-hydroxylase enzyme (21OH) deficiency, codified by *CYP21A2* gene. Newborn screening program detect CAH through 17-hydroxyprogesterone (17OHP) biochemistry test, however, the levels of 17OHP can vary due to different factors, causing false positives or false negatives results.

Therefore, this work aimed to employ molecular methodologies to mutation detection of *CYP21A2* gene in suspected CAH children from southern Brazil.

METHODS

Blood samples were collected from 166 children for molecular studies of *CYP21A2* gene in south Brazil. All of them were suspect to have CAH based on clinical manifestation and biochemical tests.



(*) Arg357Try, Leu307PhefsX6, Gln319Ter, Val238Glu, IVS2-13A/C>G, Ile173Asn, Pro31Leu, Pro454Ser, Val282Leu, Gly110ValfsX21, Arg409Cys and His63Leu.

RESULTS

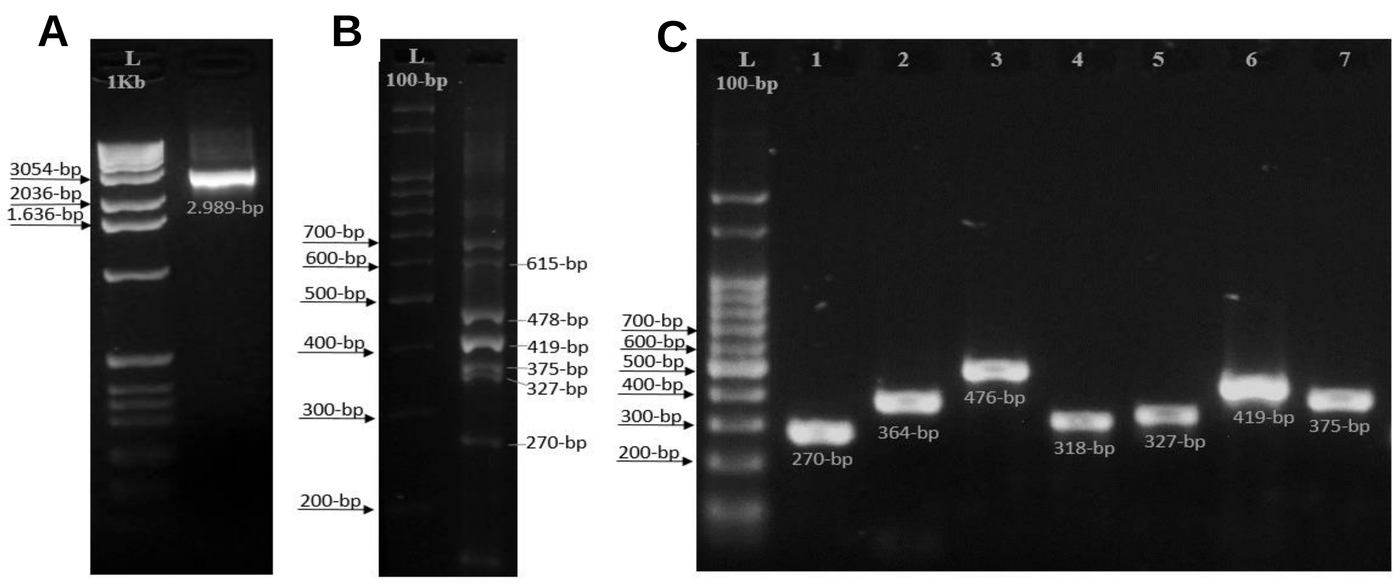


Figure 1: Electrophoresis of PCRs reactions. (A) Allele-specific PCR of *CYP21A2* gene (1% agarose gel); (B) Nested multiplex PCR amplicons (3% agarose gel) for minisequencing assay; (C) Nested PCR amplicons for Sanger sequencing assay(1.6% agarose gel). L 1 Kb: 1 Kb DNA ladder; L 100-bp: 100-bp DNA ladder.

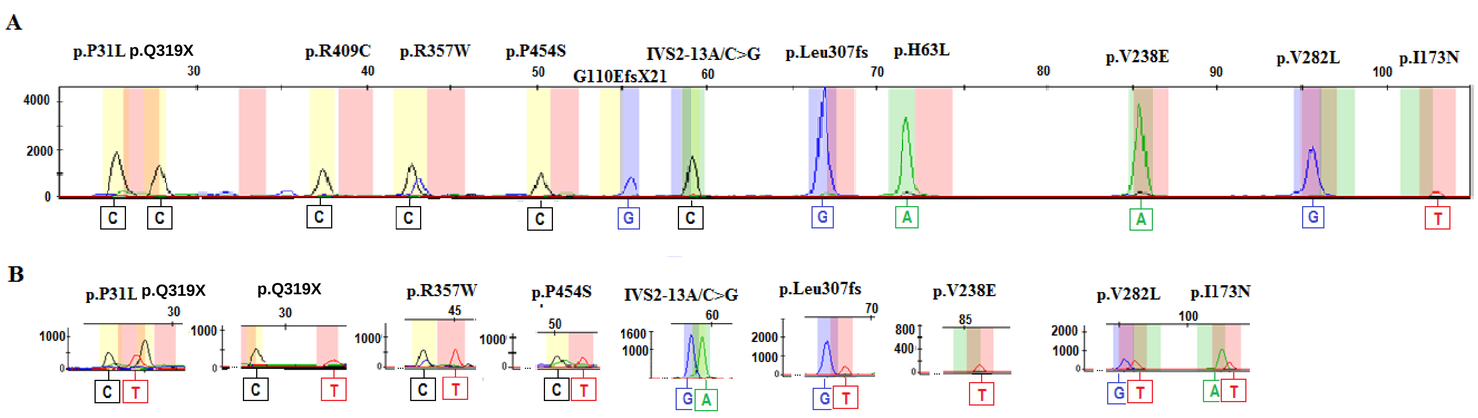


Figure 2: Electropherograms of CAH multiplex minisequencing. (A) Electropherograms of 12 wild type alleles (alleles in homozygous state). The blue peak on R357W is a reaction noise. (B) Electropherograms of 8 mutant alleles (heterozygous or homozygous state).

Table 1: *CYP21A2* genotypes grouped according to residual 21OH enzymatic activities.

Mutations groups	Genotypes	Number of Cases
Null and A groups (17)	Del CYP21A2 / Large gene conversion	1
	Del CYP21A2 / Cluster E6 (I236N and V238E)	1
	Del 30-Kb / Cluster E6 (I236N and V238E)	1
	Q319X / Q319X	2
	Del CYP21A2 / R357W	1
	Del CYP21A2 / IVS2-13A/C>G	1
	G110EfsX21; IVS2-13A/C>G / IVS2-13A/C>G	1
	IVS2-13A/C>G / IVS2-13A/C>G	6
	Del 30Kb / IVS2-13A/C>G	2
	Leu307PhefsX6; IVS2-13A/C>G / IVS2-13A/C>G	1
B group (2)	I173N /I173N	1
	Del CYP21A2 / I173N	1
C group (12)	IVS2-13A/C>G / V282L	1
	P454S / P454S	1
	Q319X; R357W; Leu307PhefsX6 / V282L	1
	Q319X / V282L	1
	R357W /V282L	1
	V282L / V282L	7
WT group (135)	Q319Ter / WT	5
	R357W / WT	4
	V282L / WT	8
	WT / WT	118

CONCLUSIONS

The methodologies used were efficient to detect the most frequently mutations in CYP21A2 *gene* and will be used in newborn screening program of Rio Grande do Sul (Brazil) to distinguish false-positive cases of ill patients.

References

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Support

CNPq

FAPERGS
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